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Full Length Article

# Role of glypican-3 immunocytochemistry in differentiating hepatocellular carcinoma from metastatic carcinoma of the liver utilizing fine needle aspiration cytology

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## KEYWORDS

Liver;  
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**Abstract** *Purpose:* Evaluation of the sensitivity and specificity of glypican3 (GPC3) in differentiating hepatocellular carcinoma (HCC) from metastatic carcinomas of the liver in cell block material. *Patients and methods:* Sixty cell blocks were prepared from liver FNAs performed in the radiodiagnosis department, National Cancer Institute, in the period between August 2011 and May 2012. Cases diagnosed as hepatocellular carcinoma, or metastatic carcinoma were included in the study. Cell block sections were stained with anti GPC-3. Sensitivity, specificity, and positive and negative predictive values, of GPC3 were calculated. The final diagnosis was based on the triple approach of clinical data, radiological findings, as well as cytomorphologic features aided by GPC-3 results. *Results:* 70% of cases were diagnosed as HCC, and 30% as metastatic carcinomas. 95.2% of HCC cases expressed GPC3. Poorly differentiated cases showed the highest GPC3 sensitivity (100%), followed by moderately differentiated cases (96.5%), while well differentiated cases expressed GPC3 in 90% of cases. 83.3% of metastatic carcinomas were negative for GPC3. In this study, sensitivity of GPC-3 in HCC was 95.2%, specificity was 83.3%, positive and negative predictive values were 93% and 88.2% respectively, and total accuracy was 91.7%.

*Conclusion:* Immunocytochemical staining for GPC3 in cell block material is a highly sensitive and specific method capable of distinguishing HCC from the vast majority of metastatic carcinomas of the liver.

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## Introduction

Hepatocellular carcinoma is the fifth most common cancer and the third most common cause of cancer-related death worldwide [1]. Although the incidence of hepatocellular carcinoma is lower in the developed Western world, the number of cases is increasing [2]. In Egypt, Cancer Pathology Registry (National Cancer Institute, Cairo University, 2003–2004) reported that

liver cancer formed 11.75% of malignancies of all digestive organs and 1.68% of total malignancies. The included liver cancer cases were mostly hepatocellular carcinoma (70.48%), hepatoblastoma (10.24%), non-Hodgkin's lymphoma (4.21%), and adenocarcinoma unspecified (9.03%) [3].

The role of FNAC in the diagnosis of liver focal lesions is well-established. Although 80% of malignant lesions of the liver can be correctly diagnosed through cytomorphological analysis and good clinico-radiologic correlation, around 20% can pose differential diagnostic problems. The distinction of moderately to poorly differentiated hepatocellular carcinoma from metastatic carcinoma may be a major problem facing cytologists and this distinction is clinically important [4].

Several immunohistochemical markers are available to assist in this differential diagnosis, each with its strength and limitations, making their judicious use imperative in biopsies with limited material. The current available immunocytochemical markers, such as alpha-fetoprotein, hepatocyte antigen (Hep-Par 1), polyclonal carcinoembryonic antigen, CD10, and CD34, have significant diagnostic limitations [5].

Glypican-3 (GPC3) is one of the recent markers that had been added to the hepatocellular phenotype listing [6]. GPC3 is a 60 kDa cell-surface protein, which is a member of the heparan sulfate proteoglycan family (GPC1 to GPC6). It is an oncofetal protein that is expressed in the embryo and is involved in morphogenesis and growth control during development [7]. The gene encoding GPC3 protein has been mapped to Chromosome Xq26. Several studies have underlined an important role of GPC3 in the regulation of cell growth, differentiation, and migration [8].

Normally, GPC3 is expressed in fetal tissues and trophoblastic cells. Its expression is silenced in adult tissues [7], except for normal ovarian, mammary, and mesothelial tissues [9]. Down regulation of GPC3 is frequently detected in ovarian carcinoma, breast cancer, and mesothelioma [7], implying GPC3 as a tumor suppressor gene in these organs. In contrast, GPC3 may act as an oncofetal protein in carcinomas of various other organs, such as hepatocellular carcinoma (HCC) [10], fibrolamellar HCC [11], germ cell tumors [12], bronchogenic squamous cell carcinoma [13], and gastric cancer, as this gene is highly expressed in tumor lesions compared with corresponding normal tissues [14].

GPC3 was found to be expressed in the majority of cases of HCC with a sensitivity ranging from 72% to 90% [15], and a specificity between 96% and 100%. It has also been suggested that poorly differentiated hepatocellular carcinomas are more likely to express GPC-3 [16]. Its positivity is cytoplasmic, membranous, and canalicular [6].

The current study aimed at assessing the role of glypican3 immunocytochemistry in differentiating hepatocellular carcinoma from metastatic carcinoma of the liver, in terms of sensitivity, specificity, positive and negative predictive values.

## Patients and methods

The current study included 60 cases with malignant epithelial liver tumors diagnosed by image-guided FNAC. The fore-mentioned cases were selected from patients with hepatic focal lesions referred to the radio-diagnosis department, and registered at the cytopathology unit, pathology department,

National Cancer Institute, Cairo University, during the period from August 2011 to May 2012.

Eligibility criteria for inclusion in the study were as follows:

1. Malignant epithelial liver tumors, diagnosed as either hepatocellular carcinoma or metastatic carcinoma.
2. Adequate cytological material for primary light microscopic evaluation.
3. Availability of aspirate sufficient for cell-block preparation.

Files of the patients were reviewed and relevant clinical and radiological data were recorded. FNAC from liver focal lesions was performed under ultrasonography or computerized tomography guidance depending on size, site and number of the focal lesions using a 23-gauge spinal needle. Six to eight smear slides and one cell block were prepared for each case. The slides were immediately fixed in 95% ethyl alcohol for a minimum of 15 min, and stained with modified Papanicolaou stain. After careful screening of the slides prepared for each case, malignant cases were selected, which were further classified, if possible, into primary (HCC) or secondary (metastatic carcinoma). Smears with inadequate cellularity or bad quality were considered inadequate, and excluded from the study.

Cell blocks were made by allowing bloody material to clot, whether on a slide or inside the syringe, scrapping the clotted material into 10% neutral buffered formalin, and then processing by routine histopathologic technique [17].

For each case, 5- $\mu$ m section was cut and the slide was stained with hematoxylin and eosin, all cell blocks were evaluated for cellular adequacy. Additional section was prepared for each case on an electrostatically charged glass slide, and stained with monoclonal mouse, anti-glypican-3 antibody concentrate (Cell Marque, USA), clone 1G12. The labeled streptavidin-biotin immunoperoxidase technique (LSAB) was used. The reaction was detected using Diaminobenzidine (DAB), with a hydrogen peroxide ( $H_2O_2$ ) block. Positive control slides were run with each staining set to ensure that all reagents were working properly. Negative control slides, were performed by substituting Phosphate Buffered Saline (PBS) for the primary antibody, and were processed parallel with each staining set to evaluate non-specific staining and better interpretation of specific staining at the antigen site. All slides were counterstained with hematoxylin, and examined for GPC-3 expression. Glypican-3 staining intensity and pattern were graded according to a 4-tier system as negative (grade 0), weak cytoplasmic staining (grade 1), moderate cytoplasmic staining (grade 2), and strong cytoplasmic staining with membranous accentuation (grade 3) [9].

For statistical analysis, data were coded and entered using the statistics package SPSS version 15. Chi-square and Fisher-exact tests were used for testing proportion independence.

Validity of glypican-3 immunostaining in diagnosing hepatocellular carcinoma and differentiating it from metastatic carcinoma, was done by calculating; sensitivity, specificity, positive predictive value, negative predictive value, and total accuracy.

## Results

The present study was conducted on 60 patients presented with hepatic focal lesions, and diagnosed as malignant epithelial tumors on the basis of fine needle aspiration cytology. Cases

were classified into hepatocellular carcinoma, and metastatic carcinoma. HCC represented the majority of studied cases 42/60 (70%), with metastatic carcinomas constituting 18/60 (30%). The 18 cases of metastatic carcinomas were categorized, on the basis of available previous clinical history, as pancreatic adenocarcinoma 5/18 (27.8%), gastric adenocarcinoma 1/18 (5.5%), non-small cell lung cancer 1/18 (5.5%), chromophobe renal cell carcinoma 1/18 (5.5%), and colorectal adenocarcinoma 2/18 (11%). Eight out of 18 cases (44.7%) of metastatic carcinoma showed cytomorphologic features of metastatic adenocarcinoma but had no settled diagnosis of the primary tumor yet. These cases were categorized as metastatic adenocarcinoma, not otherwise specified (NOS) (Table 1).

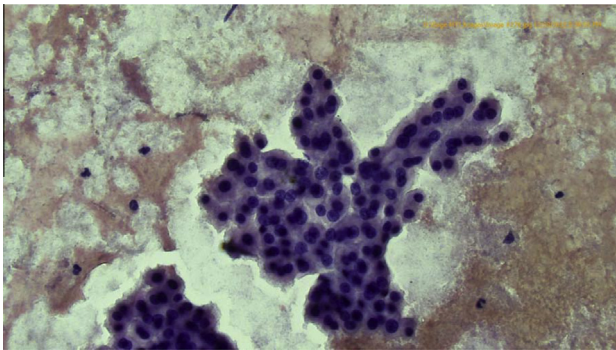
Male predominance was observed in the studied cases in both HCC and metastatic carcinoma with male to female ratio of 9.5:1 and 1.25:1 respectively. The age range of the studied cases was from 24 to 79 years, with a mean age of 62.5 years in hepatocellular carcinoma with a standard deviation of 9.03, and 58.2 years in metastatic carcinoma group with a standard deviation of 11.52.

The majority of the studied cases of hepatocellular carcinoma (71.4%) presented radiologically with multiple hepatic focal lesions, and only 29.6% of cases were solitary. On the other hand, metastatic carcinoma cases presented principally by multiple hepatic focal lesions (72.2%).

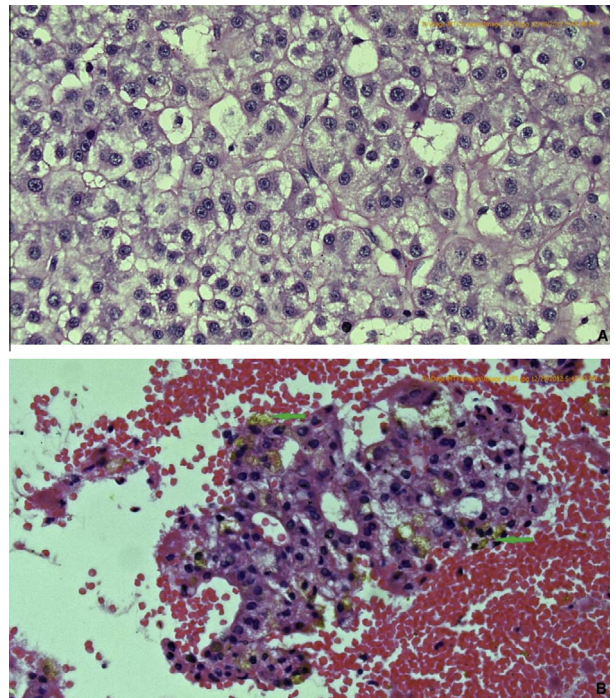
The cytomorphologic analysis encompassed the study of cellularity, pattern of arrangement, cytoplasmic and nuclear details and many additional features. The useful cytological features in the diagnosis of HCC include trabecular pattern with small capillaries transgressing or wrapping clusters of tumor cells, hepatocytic cells with abundant eosinophilic granular cytoplasm, cytoplasmic bile pigment, intranuclear cytoplasmic inclusions, prominent nucleoli, and atypical stripped nuclei. Based on the pattern of arrangement and nuclear features, cases of HCC were classified into three groups: (i) well differentiated, 10/42 cases (23.8%), (ii) moderately differentiated, 29/42 cases (69%), and (iii) poorly differentiated, 3/42 cases (7.2%) (Figs. 1, 2 and 4–6 and Table 2).

In the current work, glypican-3 (GPC-3) staining intensity and pattern were graded according to a 4-tier system as (0, 1, 2, and 3) grades [9]. Glypican3 immunoreactivity was cytoplasmic with sometimes membranous and occasionally perinuclear accentuation. Forty out of 42 cases of HCC gave positive immunocytochemical reaction to GPC3 (95.2%), while only 3 out of 18 cases (16.7%) of metastatic carcinoma showed positive reaction, with a *P* value <0.001 that is statistically significant (Table 3).

Two of 42 samples (4.8%) from the HCC group patients were graded 0, and 40 of 42 (95.2%) samples were graded 1,



**Figure 1** Well-differentiated hepatocellular carcinoma showing monotonous population of small atypical hepatocytes in trabeculae that are three cells thick or more (Papanicolaou ×400).

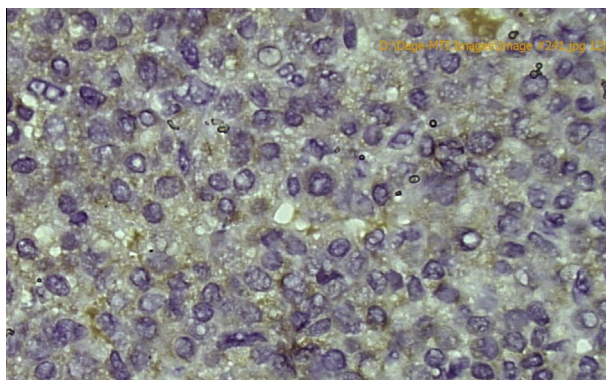


**Figure 2** Well-differentiated HCC, in cell block preparations: (A) cords of malignant hepatocytes, which exhibit well defined cell borders, ample granular cytoplasm, central round nucleus with distinct nucleolus and granular chromatin (H&E ×400). (B) The pseudoacinar pattern, with acinar-like structures lined by small atypical hepatocytes, with intracytoplasmic greenish brown bile pigment (green arrows) (H&E ×400).

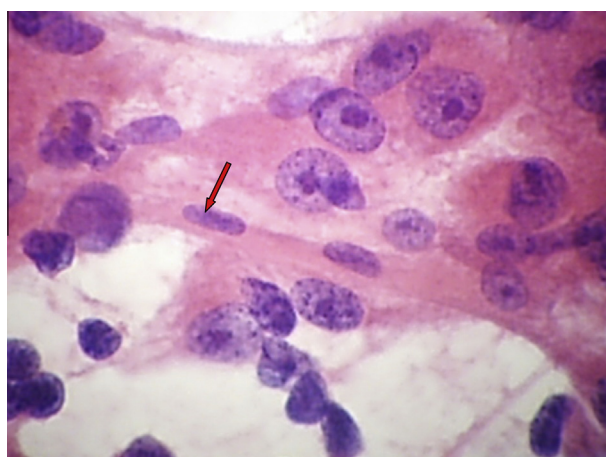
**Table 1** Different cytologic diagnoses of studied cases.

	Cytologic diagnosis						
	HCC	Metastatic carcinoma					
		Pancreatic carcinoma	Gastric carcinoma	Non-small cell lung	Chromophobe renal cell carcinoma	Colorectal carcinoma	Metastatic adenocarcinoma NOS
No. of cases	42	5	1	1	1	2	8
Total	42	18					

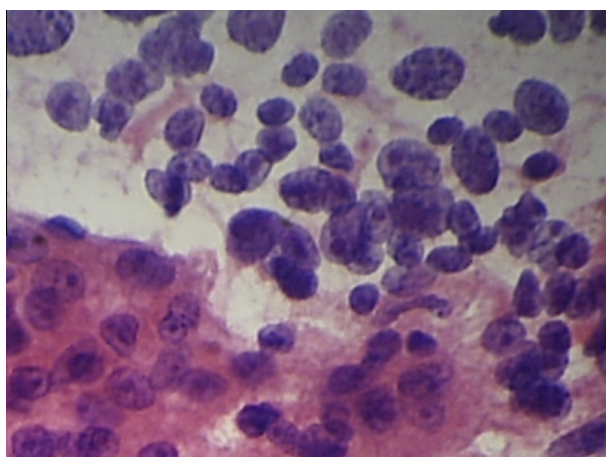




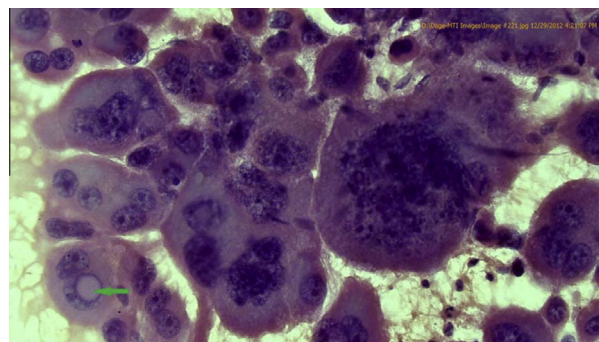
**Figure 3** Well differentiated HCC showing weak cytoplasmic staining for GPC-3 (grade1) (GPC3  $\times 400$ ).



**Figure 4** Moderately differentiated hepatocellular carcinoma, showing malignant hepatocytes with abundant eosinophilic granular cytoplasm, coarse granular chromatin, prominent nucleoli, and sinusoidal endothelial cells (red arrow) (Papanicolaou  $\times 1000$ ).



**Figure 5** Moderately differentiated HCC, showing pleomorphic atypical hepatocytes with numerous naked nuclei having the same features as cells in the trabeculae (Papanicolaou  $\times 400$ ).



**Figure 6** Poorly differentiated HCC, showing pleomorphic tumor giant cells with bizarre nuclei. Although, it still shows focal hepatocellular differentiation; prominent nucleoli and intranuclear inclusions (arrow) (Papanicolaou  $\times 400$ ).

**Table 2** Different grades of differentiation among the studied cases of hepatocellular carcinoma.

Grade of differentiation	Hepatocellular carcinoma	
	No.	Percent
Well-differentiated	10	23.8
Moderately-differentiated	29	69
Poorly-differentiated	3	7.2
Total	42	100

2 or 3; 1 case was graded 1 (2.4%), 22 cases were graded 2 (55%), and 17 cases were graded 3 (42.5%) (Figs. 3 and 7–9). Among the patients of the metastatic carcinoma group, 15/18 (83.3%) samples were graded 0, and 3/18 (16.7%) samples were graded 2 (Figs. 10–13). *P* value was calculated to be  $< 0.001$  that is statistically significant (Table 4).

GPC-3 was found to be expressed in the majority of cases of HCC with a sensitivity (95.2%), specificity (83.3%), positive predictive value (PPV) (93%), negative predictive value (NPV) (88.2%) and total accuracy of 91.7%.

## Discussion

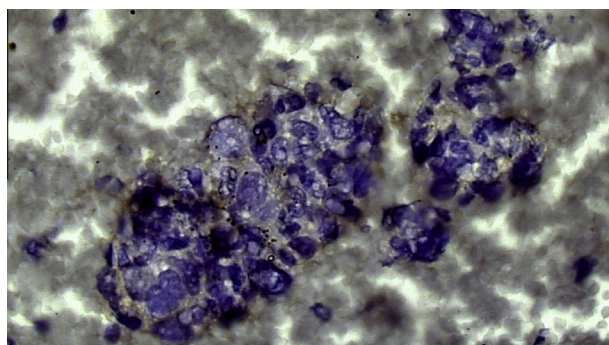
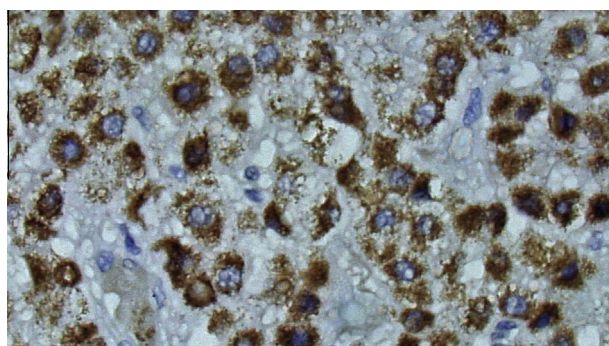
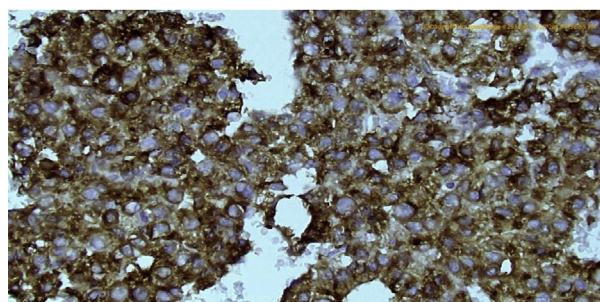
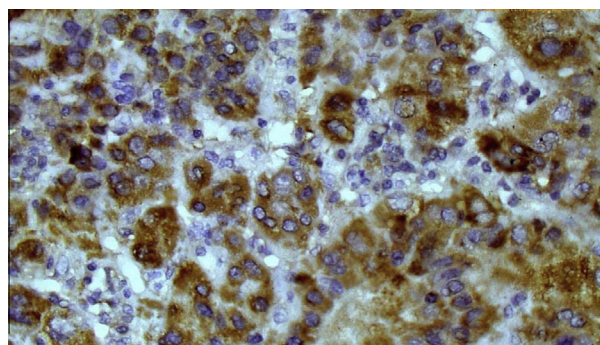
Hepatocellular carcinoma is one of the most common cancers in the world, with an estimated incidence of 1,000,000 cases per year [18]. Recent reports demonstrate that the incidence of HCC has increased sharply in the last 5–10 years [19], with an especially high incidence in Egypt [20].

Metastatic tumors account for most hepatic malignant diseases in non-cirrhotic livers in developed Western countries. In the cirrhotic liver, however, primary hepatic malignant tumors are more common than are metastatic tumors [21]. The distinction of hepatocellular carcinoma from other neoplasms involving the liver can be difficult and is especially challenging in core and fine-needle aspiration biopsies.

Preliminary studies have demonstrated the promise of GPC3 immunohistochemistry for the diagnosis of hepatocellular carcinoma. It has also been suggested that poorly differentiated hepatocellular carcinomas are more likely to express GPC3 [16]. It may be very helpful in differentiating HCC from benign hepatic lesions and from metastatic neoplasms of the liver [22].

**Table 3** Glypican-3 immunostaining results among studied cases.

Glypican-3 immunostaining	Hepatocellular carcinoma		Metastatic carcinoma	
	No.	Percent	No.	Percent
Positive	40	95.2	3	16.7
Negative	2	4.8	15	83.3
Total	42	100	18	100

**Figure 7** Moderately differentiated HCC showing negative staining for GPC3 (grade 0) (GPC3 ×400).**Figure 8** Moderately differentiated HCC showing moderate cytoplasmic staining for GPC-3 (grade 2) (GPC3 ×400).**Figure 9** Two cases of moderately differentiated hepatocellular carcinoma showing strong cytoplasmic staining for GPC-3 with membranous accentuation (grade 3) (GPC-3 ×400).

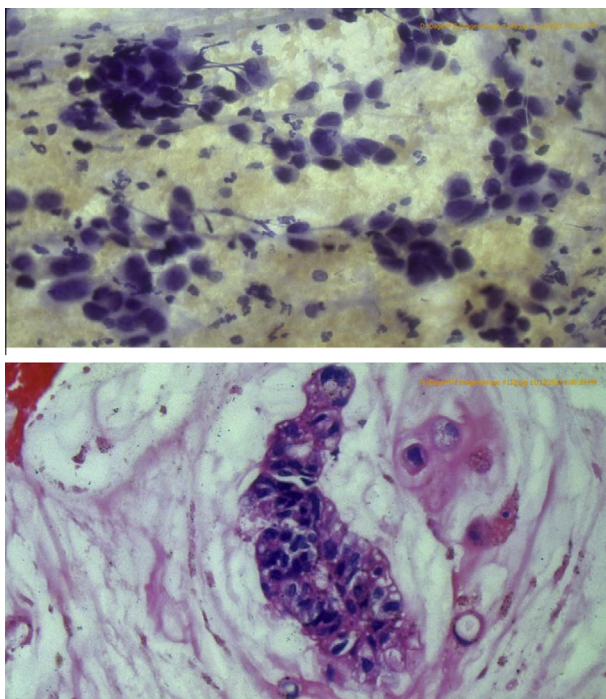
This study was conducted on 60 cases of focal liver lesions diagnosed on the basis of fine needle aspiration cytology. The final diagnosis for each case was achieved on the basis of the triple approach of: (1) radiological findings, (2) clinical data including tumor marker serum level (alpha-fetoprotein and others related to primary tumors elsewhere), and a history of any primary carcinoma that could metastasize to the liver, as well as (3) cytomorphologic criteria aided by the GPC-3 immunocytochemical result.

In the current work, 42 out of 60 cases (70%) of liver focal lesions were diagnosed as HCC, representing the majority of our studied cases, with metastatic carcinomas constituting 18 out of 60 cases (30%). This finding agrees with what Ligato et al. (2008) have recorded that 58.5% of malignant hepatic tumors were HCC (representing the majority of cases) and 41.5% were metastatic in origin [22].

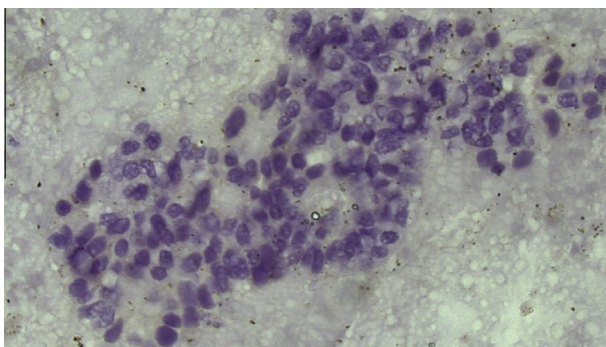
The 18 cases of metastatic carcinomas were categorized as 5/18; pancreatic adenocarcinoma (27.8%), 1/18; gastric cancer

(5.5%), 1/18; non-small cell lung cancer (5.5%), 1/18; chromophobe renal cell carcinoma (5.5%), 2/18; colorectal cancer (11%), and 8/18; metastatic adenocarcinoma, not otherwise specified (NOS) (44.7%). In metastatic cases, specification of the primary site was based on clinical history. Cases diagnosed as metastatic adenocarcinoma (NOS) have no settled diagnosis of primary tumor elsewhere. These results agree with what was reported in a previous study concerning liver metastasis that lung, colon, pancreas, breast, and stomach are the most frequent primary sites of origin for metastatic carcinoma of liver, representing 24.8%, 15.7%, 10.9%, 10%, and 6.1% of cases, respectively [23]. However, the higher percentages of these primary sites in the mentioned study than the current study (with the exception of pancreatic adenocarcinoma) could be attributed to the dependence of most clinicians in a large number of patients presenting with focal liver lesions and with a known previous history of primary cancer elsewhere on correlation of radiological data, clinical history and tumor marker serum level without necessitating tissue diagnosis for managing these cases as metastatic. Another explanation is the higher presentation of cases of metastatic adenocarcinoma (NOS) over other categories of metastatic carcinoma in the current work.

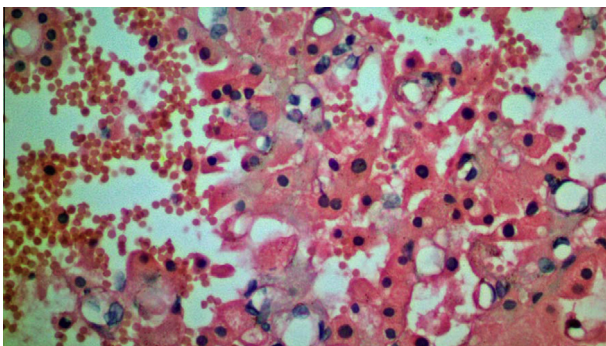




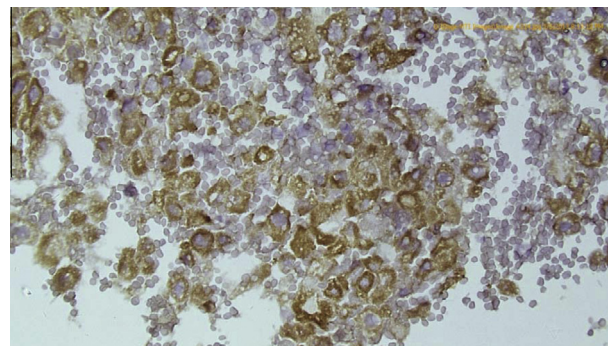
**Figure 10** Fine needle aspiration of metastatic adenocarcinoma in both direct smear (upper) (Papanicolaou stain  $\times 400$ ), and cell block (lower) (H&E  $\times 400$ ).



**Figure 11** Negative glypican-3 immunostaining of metastatic adenocarcinoma (grade 0) (GPC-3  $\times 400$ ).



**Figure 12** Cell block preparation of metastatic chromophobe renal cell carcinoma showing sheets of polygonal cells, with well-defined cell borders, and dense granular cytoplasm (H&E  $\times 400$ ).



**Figure 13** Glypican-3 immunostaining of metastatic chromophobe renal cell carcinoma, showing moderate cytoplasmic staining for GPC-3 (grade 2) (GPC-3  $\times 400$ ).

In the included cases, HCC occurred more frequently in cirrhotic livers (83.5%), than in non-cirrhotic livers (16.5%). This agrees with what is reported in the literature of the higher frequency of hepatocellular carcinoma cases (58%) in cirrhotic livers [24].

The majority of the studied cases of hepatocellular carcinoma (71.4%) presented radiologically with multiple hepatic focal lesions, and only 29.6% of cases were in the form of solitary hepatic focal lesions. The multicentric form of hepatocellular carcinoma was also reported by Craig [25], to represent the majority of hepatocellular carcinoma cases. On the contrary, Ahuja et al. (2007) reported that most of hepatocellular carcinoma cases (68%) presented as solitary hepatic focal lesions [25,4]. This discrepancy could be attributed to variations between different countries in the main implicated carcinogenic agents that give different HCC radiological presentations. On the other hand, metastatic carcinoma cases presented principally by multiple hepatic focal lesions (72.2%), and this agrees with Ahuja et al. (2007) who observed that 68% of metastatic carcinoma cases presented by multiple hepatic focal lesions [4].

Cytomorphologically, according to the pattern of arrangement and nuclear features, the studied cases of HCC were classified into three groups: (i) well differentiated, 10/42 cases (23.8%), (ii) moderately differentiated, 29/42 cases (69%), and (iii) poorly differentiated, 3/42 cases (7.2%).

After careful cytomorphologic examination of cases a provisional diagnosis was given in each case, GPC3 immunocytochemistry was carried out to complete the diagnostic picture.

In the current work, two of 42 samples (4.8%) from the HCC group were graded 0, and 40 of 42 samples (95.2%) were graded 1, 2 or 3; 1 case was graded 1 (2.4%), 22 cases were graded 2 (52.4%), and 17 cases were graded 3 (40.5%). These findings are slightly higher than what have been reported by Kandil and Cooper (2007), and Ligato et al. (2008) ; 90% and 83.3 of HCC cases showed positive immunoreactivity for glypican-3, respectively [9,22].

In contrast, among the patients of metastatic carcinoma group, 15/18 (83.3%) samples were graded 0, and 3/18 (16.7%) samples were graded 2. Our findings were slightly higher than what is reported in the literature regarding GPC-3 positivity in metastatic carcinoma cases that ranged from 0% to 5.9% [9,22,26].

**Table 4** Glypican-3 staining intensity grades among the studied cases.

Glypican-3 immunostaining intensity	Hepatocellular carcinoma		Metastatic carcinoma	
	No.	Percent	No.	Percent
Grade 0	2	4.8	15	83.3
Grade 1	1	2.4	0	0
Grade 2	22	52.4	3	16.7
Grade 3	17	40.5	0	0
Total	42	100	18	100

The three cases which were positive for glypican-3 in the metastatic carcinoma group include: (1) metastatic chromophobe renal cell carcinoma, which showed diffuse moderately-intense staining (Figs. 12 and 13). This unexpected result matched with what was published by Okoń (2008); where glypican-3 immunohistochemistry was performed on 625 cases of renal cell carcinoma, revealing strong positive staining in 15 cases, moderate in 4 and weak in 68. The reactivity was particularly evident in chromophobe renal cell carcinoma (32/40) [27], (2) metastatic pancreatic adenocarcinoma, which showed focal moderately-intense positive staining. This finding was also reported by Yan et al. (2011); the primary adenocarcinomas of the pancreas (1/17; 6%) have displayed focal glypican-3 immunoreactivity [28], and (3) metastatic colorectal adenocarcinoma, which showed focal moderately-intense positive staining. This finding was also reported by Yamauchi et al. (2005) who achieved similar results where one case of colorectal carcinoma exhibited focal positivity for GPC-3 [26].

In our study, GPC-3 was found to be expressed in the majority of cases of HCC with a sensitivity (95.2%), specificity (83.3%), positive predictive value (PPV) (93%), negative predictive value (NPV) (88.2%) and total accuracy of 91.7%. Other studies showed sensitivity ranging from 72% to 90%, specificity between 96% and 100%, positive predictive value of 95%, and negative predictive value of 85.7% [15,16,22].

It has to be noted that there is some risk of sampling problems and consequently, negative results do not always exclude the diagnosis of HCC. On the other hand, we should be cautious to interpret a positive immunostaining for GPC3 as a definitive proof for a diagnosis of HCC. This is because of the reported focal expression of GPC3 in dysplastic nodules, and in rare cases of metastatic carcinomas to the liver [22]. Therefore, it is mandatory to interpret a positive immunostaining result for GPC3 within the appropriate clinical, radiological, and cytomorphologic context.

Finally, we conclude that immunocytochemical staining for GPC3 using FNA cell block material is a highly sensitive and specific method capable of distinguishing HCC from the vast majority of metastatic carcinomas of the liver.

#### Conflict of interest statement

I declare that there is no conflict of interest.

#### References

- [1] El-Serag HB. Hepatocellular carcinoma: an epidemiologic view. *J Clin Gastroenterol* 2002;35(5 Suppl. 2):572–8.
- [2] Tanaka Y, Hanada K, Mizokami M, Yeo AE, Shih JW, Gojobori T, et al. A comparison of the molecular clock of hepatitis C virus in the United States and Japan predicts that hepatocellular carcinoma incidence in the United States will increase over the next two decades. *Proc Natl Acad Sci USA* 2002;99:15584–9.
- [3] Mokhtar N, Gouda I, Adel I. Cancer pathology registry (2003–2004) and time trend analysis. Egypt: Department of Pathology, National Cancer Institute, Cairo University; 2007.
- [4] Ahuja A, Gupta N, Srinivasan R, Kalra N, Chawla Y, Rajwanshi A. Differentiation of hepatocellular carcinoma from metastatic carcinoma of the liver – clinical and cytological features. *J Cytol* 2007;24(3):125–9.
- [5] Wee A. Diagnostic utility of immunohistochemistry in hepatocellular carcinoma, its variants and their mimics. *Appl Immunohistochem Mol Morphol* 2006;14:266–72.
- [6] Desmet VJ, Rosai J. Liver. In: Rosai J, editor. *Rosai and Ackerman's surgical pathology*. Elsevier Inc.; 2011. p. 857–942.
- [7] Zou ZQ, Ding YP, Long B, Yuh JG, Xu AL, Lang ZW, et al. Gpc-3 is a notable diagnostic, prognostic and a latent targeted therapy marker in hepatocellular carcinoma. *Hepato-Gastroenterology* 2010;57:1285–90.
- [8] Filmus J, Capurro M, Rast J. Glypicans. *Genome Biol* 2008;9:224.
- [9] Kandil DH, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. *Adv Anat Pathol* 2009;16:125–9.
- [10] Coston WM, Loera S, Lau SK, Ishizawa S, Jiang Z, Wu CL, et al. Distinction of hepatocellular carcinoma from benign hepatic mimickers using glypican-3 and CD34 immunohistochemistry. *Am J Surg Pathol* 2008;32:433–44.
- [11] Ward SC, Huang J, Tickoo SK, Thung SN, Ladanyi M, Klimstra DS. Fibrolamellar carcinoma of the liver exhibits immunohistochemical evidence of both hepatocyte and bile duct differentiation. *Mod Pathol* 2010;23:1180–90.
- [12] Esheba GE, Pate LL, Longacre TA. Oncofetal protein glypican-3 distinguishes yolk sac tumor from clear cell carcinoma of the ovary. *Am J Surg Pathol* 2008;32:600–7.
- [13] Aviel-Ronen S, Lau SK, Pintilie M, Lau D, Liu N, Tsao MS, et al. Glypican-3 is overexpressed in lung squamous cell carcinoma, but not in adenocarcinoma. *Mod Pathol* 2008;21:817–25.
- [14] Yan B, Wei J, Qian Y, Zhao X, Zhang W, Xu A, Zhang S. Expression and clinicopathologic significance of glypican-3 in hepatocellular carcinoma. *Ann Diagn Pathol* 2011;15:162–9.
- [15] Wang XY, Degos F, Dubois S, Tessitore S, Allegretta M, Guttman RD, et al. Glypican-3 expression in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006;37:1435–41.
- [16] Libbrecht L, Severi T, Cassiman D, Vander Borgh S, Pirenne J, Nevens F, et al. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006;30:1405–11.
- [17] Chhieng David C, Stelow Edward B. Introduction to pancreatic cytopathology. In: Chhieng David C, Stelow Edward B, editors. *Pancreatic cytopathology*. New York, NY: Springer Science + Business Media Inc.; 2007. p. 1–21.

- [18] Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005;65:6245–54.
- [19] Poustchi H, Sepanlou SG, Esmaili S, Mehrabi N, Ansarymoghdam A. Hepatocellular carcinoma in the world and the Middle East. *Middle East J Dig Dis* 2010;2(1): 1–2.
- [20] Elattar I. Website of the National Cancer Institute, Egypt, 2003. Available from <http://www.nci.edu.eg> [accessed 2010].
- [21] Kay WM. Liver. In: Weidner N, Cote RJ, Suster S, Weiss LM, editors. *Modern surgical pathology*. Saunders, an imprint of Elsevier Inc.; 2009. p. 902–59.
- [22] Ligato S, Mandich D, Cartun RW. Utility of glypican-3 in differentiating hepatocellular carcinoma from other primary and metastatic lesions in FNA of the liver: an immunocytochemical study. *Mod Pathol* 2008;21:626–31.
- [23] Centeno BA. Pathology of liver metastases. *Cancer Control* 2006;13(1):13–26.
- [24] Anatelli F, Shang-Tian C, Yang XJ, Wang HL. Value of glypican 3 immunostaining in the diagnosis of hepatocellular carcinoma on needle biopsy. *Am J Clin Pathol* 2008;130:219–23.
- [25] Craig JR. Liver. In: Kissane JM, editor. *Anderson's pathology*. St. Louis, MO: C.V. Mosby Company; 1990. p. 1199.
- [26] Yamauchi N, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, et al. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005;18:1591–8.
- [27] Okoń K. Glypican-3 is expressed in chromophobe renal cell carcinomas. *Pol J Pathol* 2008;59(1):15–20.
- [28] Yan B, Wei J, Qian Y, Zhao X, Zhang W, Xu A, Zhang S. Expression and clinic-pathologic significance of glypican-3 in hepatocellular carcinoma. *Ann Diagn Pathol* 2011;15:162–9.